THE INFLUENCE OF AMOEBAE ON THE UPTAKE OF NITROGEN BY PLANTS IN GNOTOBIOTIC SOIL

E.T. ELLIOTT, D.C. COLEMAN* and C.V. COLE**

Natural Resource Ecology Laboratory, Colorado State University,
Fort Collins, Colorado, USA

Summary

Gnotobiotic associations of rhizosphere bacteria (*Pseudomonas*) with and without amoebae (*Acanthamoeba*) were grown with blue grama (*Bouteloua gracilis*) grass seedlings, in propylene oxide sterilized soil microcosms to test the hypothesis that when amoebae graze on bacteria plant growth and nitrogen content will increase.

Glucose (600 μg-C) and N amendments [as $(NH_4)_2SO_4$] were added with the microorganism inoculum to provide three initial levels of inorganic soil-nitrogen: low, 45; medium, 95; and high, 245 $\mu g\ NH_4^+ - N \cdot g^{-1}$ soil. Sixty days after seedling germination, soil and plant materials were sampled for levels of N and root and shoot biomass. The soils in microcosms containing amoebae with bacteria mineralized significantly (50-100%) more NH_4^+-N per gram of soil than those with bacteria alone, at the medium and high N levels. Shoot N concentration was significantly higher at all N levels when amoebae were present.

These results indicate a significant role of protozoa at

** *Phosphorus Laboratory, Science and Education Administration - Agricultural Research, U.S. Department of Agriculture, Fort Collins, Colorado, USA* the soil-root interface by accelerating the mineralization of microbially immobilized nutrients.

Introduction

Microflora play an essential role in decomposing plant residues, but compete with primary producers for the uptake of mineralized nutrients. Therefore, the release of nutrients immobilized in microflora tissues by grazers such as amoebae could play an essential role maintaining nutrient levels for plant growth. Elliott and Coleman (1977) have shown that large numbers ($1\text{-}5 \times 10^4 \cdot g^{-1}$ of surface soil) of actively-feeding, bacteriophagic protozoa exist in grasslands, even in dry soils.

Recent experiments using sterilized soil reinoculated with known organisms have helped elucidate the roles of some rhizosphere microorganisms (Coleman *et al.*, 1977). Rates of CO_2 evolution and P_i and NH_4^+ mineralization were greater in microcosms where bacteria and amoebae or bacteria and nematodes were present together than when there were only bacteria (Coleman *et al.*, 1977, 1978; Cole *et al.*, 1978; Woods *et al.*, submitted). This tends to support Johannes' concept (1965, 1968) for aquatic ecosystems of accelerated release of nutrients by bacterial grazers.

There have been many studies of interactions between protozoa and higher plants (Geltzer, 1963; Nikoljuk and Geltzer, 1972), but few controlled studies of microfauna-bacteria-plant interactions have been made; thus, Rosswall, Lohm and Sohlenius (1977) grew pine seedlings in microcosms with and without microbial grazers, with inconclusive results, due to experimental variability.

The present study tests the hypothesis that plant growth and nutrient concentration would be greater when bacteria and grazing amoebae were present together in soil than when bacteria only were present. We also hypothesized that the difference in plant response between bacteria alone and bacteria plus amoebal treatments would be greater under the most N-limiting conditions.

Materials and Methods

Gnotobiotic soil microcosms were prepared by adding 100 g of soil to 250-ml Erlenmeyer flasks. The soil was moistened to field capacity, the flasks plugged with cotton, and after incubation for two days, were sterilized with propylene oxide for 3 days. The soil lots were dried and then stored.

Bacteria were inoculated into 18 of the sterile soil microcosms and amoebae added to one-half (9) of them. Seeds were added 5 days after bacterial inoculation.

The bacteria (*Pseudomonas cepacia*) and amoebae (*Acanthamoeba polyphaga*) used in this experiment were isolated from the rhizosphere of blue grama, as described by Anderson *et al.* (1978). Microcosms were inoculated with 1 x 10^7 bacteria per gram soil in 15 ml of solution and 1 x 10^4 amoebae per gram of soil in 5 ml of solution 1 day later. Seeds of *Bouteloua gracilis* (H.B.K.) Lag. ex. Steud. were surface sterilized in a 2% solution of sodium hypochlorite for 60 minutes followed by repeated rinsing with sterile distilled water. Ten to twelve seeds were added to each microcosm under aseptic conditions.

We used an Olney clay loam (17% at field capacity) collected from the Pawnee Site, of the Natural Resource Ecology Laboratory, Colorado State University, located on the USDA Science and Education Administration Central Plains Experimental Range in north-eastern Colorado. After sterilizing, the soil contained 45 $\mu g \cdot g^{-1}$ of NH_4^+-N. Glucose (500 μg C $\cdot g^{-1}$) and 50 and 200 μg of NH_4^+-N $\cdot g^{-1}$ soil [as $(NH_4)_2SO_4$] were added with the bacterial inoculum. The amoebae were added in 5 ml of proteose-peptone, glucose, and mineral solution (PPGM) bringing the soil water to 20%. The PPGM addition, which was also pipetted into flasks with no amoebal additions, added 3 μg of inorganic

P, 70 μg of proteose-peptone-N, and 100 μg of glucose-C, all · g^{-1} soil. However, since the utilization of organic N in the PPGM was not determined, its amount was not included in the N-flow calculations.

The assembled flasks were maintained in an environmental chamber on a 12-h light (30°C) and 12-h dark cycle (22°C). Soil water was maintained with sterile distilled water. Several 1-cm diameter cores were removed from each flask, extracted with 2 M KCl, 10:1 (v/w), and assayed by the Conway microdiffusion technique (Stanford *et al.*, 1973). Plants were separated from the soil by gently washing roots in a fine mesh screen. Shoot weights were obtained after drying at 60°C for 48 h and root weights were determined as ash-free dry weights. Shoot nitrogen content was measured using a Kjeldahl digest in combination with the Conway microdiffusion technique.

The experiment was a randomized block design with each of three replicates handled as a block. Data were analyzed by an analysis of variance and all differences reported are significant at $P \leq 0.05$.

Results and Discussion

At the end of the growth period (60 days), we measured total plant yield (Table 1). Total yield (mg · $flask^{-1}$) was significantly affected by fertilizer levels but not biological interactions.

Glucose added initially with the inoculum-stimulated bacterial N uptake in agreement with previous microcosm studies without plants (Coleman *et al.*, 1977; Woods *et al.*, in preparation). Amoebal activity was evident, since at the end of the growth period there was significantly more soil NH_4^+-N in the presence of amoebae (AB treatment) at the two higher N levels than with bacteria alone (B treatments) (Fig. 1) (23.1 vs. 10.6 ppm and 145.1 vs. 111.6 ppm, respectively). The nitrogen content of shoots was increased when amoebae were present as compared to bacteria

TABLE 1

Mean plant yields per flask as mg dry matter (±SD) in the presence of bacteria (B), or bacteria and amoebae (AB)

	N level	Low	Medium	High
Shoot production	B	12.6 ± 2.5	21.7 ± 1.1	31.4 ± 3.5
	AB	11.8 ± 2.2	23.9 ± 4.6	25.3 ± 5.3
Root production	B	7.1 ± 1.0	11.0 ± 1.2	15.4 ± 3.4
	AB	5.9 ± 1.6	10.8 ± 3.3	11.3 ± 1.7
Total plant production	B	19.7 ± 3.1	32.7 ± 2.0	46.8 ± 6.9
	AB	17.7 ± 3.7	34.7 ± 7.6	36.6 ± 6.6

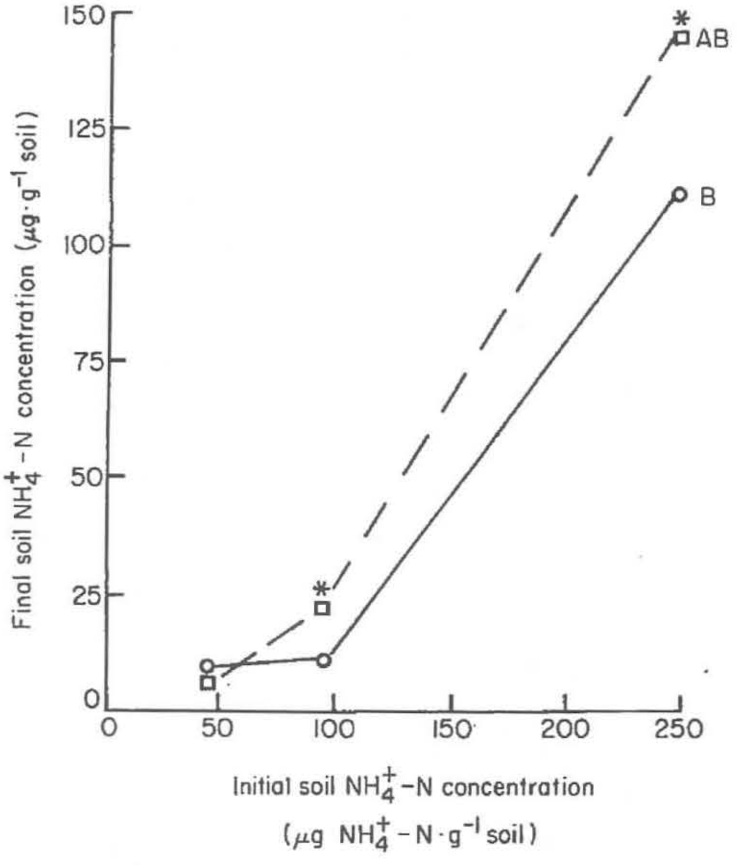

Fig. 1. The soil NH_4^+-N concentration in flasks at the end of the growth period with bacteria alone (B) or with amoebae (AB) at three initial soil NH_4^+-N concentrations. * Significant at $P < 0.05$.

alone at all three N levels (Fig. 2). The percentage N in shoots ranged from 0.8% for the low N, and bacteria alone treatment to 2.3% for the high N amoebae and bacteria treatment. In contrast, plant N uptake showed fewer dif-

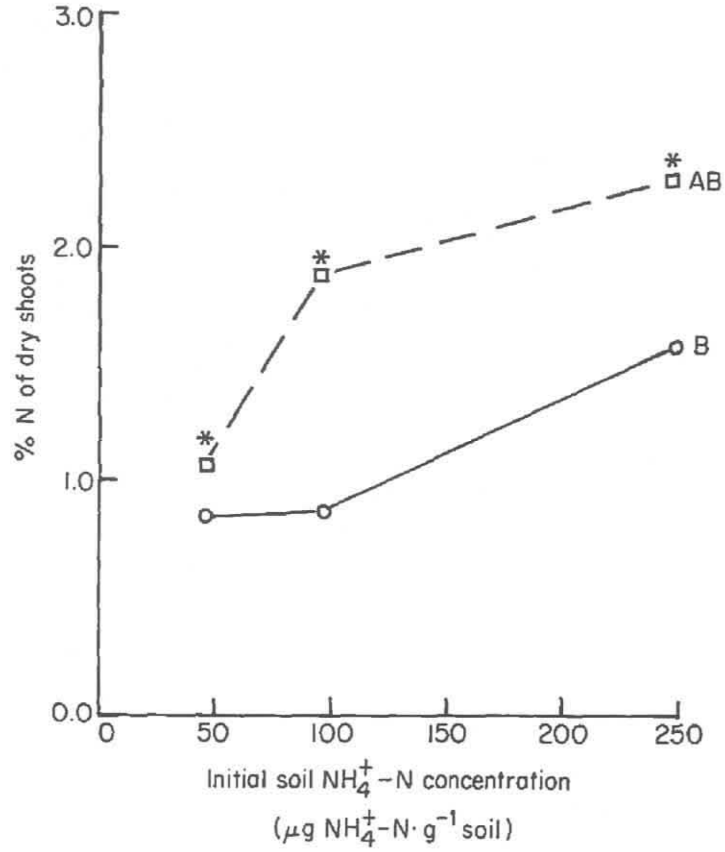

Fig. 2. The per cent N of dry shoots grown in soil in flasks with bacteria alone (B) or with amoebae (AB) at three initial soil NH_4^+-N concentrations. * Significant at $P < 0.05$.

ferences (Table 2) with only the medium N treatment showing significantly raised N levels with amoebae when contrasted to the bacteria-alone controls.

The percentage of net NH_4^+-N uptake by plants (Table 3) was calculated using the equation:

$$\%\ \text{net uptake by plants} = \frac{PSNC \cdot (RW + SW) \cdot 100}{N_i - N_f}$$

where PSNC is the plant shoot nitrogen concentration ($\mu g \cdot g^{-1}$ dry wt), RW and SW are root and shoot weight ($\cdot\ g^{-1}$ soil), N_i and N_f are the initial and final soil NH_4^+-N level ($\mu g\ NH_4^+\text{-}N \cdot g^{-1}$ soil). There were no differences between the biological treatments at the low N level but at the medium and high levels plants had a greater per cent net uptake with amoebae.

TABLE 2

Total plant uptake of nitrogen (mg · g^{-1} soil ± SD) as affected by amoebae at different nitrogen levels. (B) bacteria, (AB) amoebae and bacteria present

N level	Low	Medium	High
B	1.65 ± 0.41	2.83 ± 0.24	7.27 ± 1.10
AB	1.93 ± 0.56	6.57 ± 1.40	8.37 ± 1.43

TABLE 3

The effect of amoebal presence on plant N uptake relative to total N uptake by plants and bacteria (± SD) (expressed in per cent). (B) bacteria, (AB) amoebae and bacteria present

N level	Low	Medium	High
B	4.63 ± 1.21	3.37 ± 0.31	5.40 ± 0.57
AB	4.87 ± 1.47	9.10 ± 1.55	8.47 ± 1.79

C/N/P ratios have been used to predict rates of mineralization by bacterial predators (Hunt *et al.*, 1977). The mechanisms for mineralization have been based on the assumptions that bacteria and amoebae have similar C/N ratios. The incorporation of C and N into amoebal tissue and the loss of amoebal respiratory CO_2 forces the excretion of N containing material with a narrower C/N ratio than the bacteria consumed by amoebae. The released NH_4^+-N may be reimmobilized by bacteria or plant roots. Since bacteria are better competitors for NH_4^+ than roots (Goring and Clark, 1948), bacteria will take up a larger portion of available N. When C sources are depleted in the soil, bacteria may be less efficient at NH_4^+ uptake and roots will remove a greater proportion of available N.

Our second hypothesis, which asserted plant N growth responses would be greatest at low N levels, was not supported. Our soil N data showed that NH_4^+-N was low in both biotic treatments (Fig. 2) so plant N uptake did not differ. At the high N level there was a large difference in final soil NH_4^+-N, but as the NH_4^+ in both amoebae-bacteria and bacteria treatments was beyond plant needs, perhaps the difference was inconsequential. However, the plant N production (Table 2) was sensitive to the differences in soil N at the intermediate level, as influenced by the two biotic treatments.

Final soil NH_4^+-N, per cent N of dry shoots, plant N production, and the proportion of the net NH_4^+-N uptake attributed to plants all benefited by amoebal presence. These results indicate a significant role for protozoa at the soil-root interface by accelerating the mineralization of microbially immobilized nutrients.

Acknowledgements

We thank M. Campion and A. Rubink for extensive statistical and technical assistance. This study was supported by NSF Grant DEB-7513468 A02.

References

Anderson, R.V., Elliott, E.T., McClellan, J.F., Coleman, D.C., Cole, C.V. and Hunt, H.W. (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. III. Biotic interactions of bacteria, amoebae, and nematodes. *Microb. Ecol.*, 4 (in press).

Cole, C.V., Elliott, E.T., Hunt, H.W. and Coleman, D.C. (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. V. Phosphorus transformations. *Microb. Ecol.*, 4 (in press).

Coleman, D.C., Cole, C.V., Anderson, R.V., Blaha, M., Campion, M.K., Clarholm, M., Elliott, E.T., Hunt, H.W., Schaefer, B. and Sinclair, J. (1977). An analysis of rhizosphere-saprophage interactions in terrestrial ecosystems. Soil Organisms as Components of Ecosystems. *Ecol. Bull. (Stockholm)*, 25, 299.

Coleman, D.C., Anderson, R.V., Cole, C.V., Elliott, E.T., Woods, L. and Campion, M.K. (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon. *Microb. Ecol.*, 4 (in press).

Elliott, E.T. and Coleman, D.C. (1977). Soil protozoan dynamics in a shortgrass prairie. *Soil Biol. Biochem.*, 9, 113.

Geltzer, J.G. (1963). On the behaviour of soil amoebae in the rhizospheres of plants. *Pedobiologia*, 2, 249.

Goring, C.A.I. and Clark, F.E. (1948). Influence of crop growth on mineralization of nitrogen in the soil. *Soil Sci. Soc. Am. Proc.*, 13, 261.

Hunt, H.W., Cole, C.V., Klein, D.A. and Coleman, D.C. (1977). A simulation model for the effect of predation on bacteria in continuous culture. *Microb. Ecol.*, 3, 259.

Johannes, R.E. (1965). Influence of marine protozoa on nutrient regeneration. *Limnol. Oceanogr.*, 10, 434.

Johannes, R.E. (1968). Nutrient regeneration in lakes and oceans. *In* "Advances in the Microbiology of the Sea", Vol. I (Eds. M.R. Droop and E.J.F. Wood), p.203. Academic Press, London.

Nikoljuk, V.F. and Geltzer, J.G. (1972). "Soil Protozoa of the USSR". 'FAN' Press, Tashkent, Uzbek SSR. 320pp.

Rosswall, T., Lohm, U. and Sohlenius, B. (1977). Développement d'un microcosme pour l'étude de la minéralization et de l'absorption radiculaire de l'azote dans l'humus d'une forêt de coniferes *(Pinus sylvestris* L.*)*. *Lejeunia*, 84, 1.

Stanford, G., Carter, J.N., Simpson, E.C., Jr. and Schwaninger, D.E. (1973). Nitrate determination by a modified Conway microdiffusion method. *J. Assoc. Off. Anal. Chem.*, 56, 1365.

Woods, L., Cole, C.V., Elliott, E.T., Anderson, R.V. and Coleman, D.C. (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. VI. Nitrogen transformations. *Microb. Ecol.*, (submitted).